foam stability of 93 min which satisfied only the former requirement. The second extract had a foaming power of 1.32 and the foam stability of 720 min which satisfied both requirements for foam CCC. Then, both samples were subjected to foam CCC.

Figure 9A shows a typical HPLC chromatogram of an extract from cyanobacteria bloom sample 917S containing microcystins. As indicated in the chromatogram, peaks 1 (microcystin RR), 2 (microcystin YR), 3 (microcystin LR), and 4 (microcystin LR-s) were chosen to evaluate their foam enrichment. In the first extract, the enriched concentrations of the components are only 3–4 times and polar components with retention times shorter than that of microcystin RR were still present in the foam fraction. The HPLC analysis of foam fraction and liquid fraction of the second extract is shown in **Figures 9**B and C. The enrichment reached 10–30 times and polar components are eliminated from the foam fraction indicating that the target compounds are selectively enriched. The HPLC analysis of liquid fraction of both extracts showed similar profiles. These results indicate that these foaming parameters can be effectively applied to a crude mixture containing a large amount of impurities.

Conclusions

Foam CCC can be successfully applied to a variety of samples having foam affinity with or without surfactants. The present method offers important advantages over the conventional foam separation methods by allowing efficient chromatographic separation of sample in both batch loading and continuous feeding. We believe that the foam CCC technique has a great potential in enrichment, stripping and isolation of foam-active components from various natural and synthetic products in both research laboratories and industrial plants.

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See also: **II/Chromatography: Liquid:** Countercurrent Liquid Chromatography. **III/Antibiotics:** High-Speed Countercurrent Chromatography; Liquid Chromatography; Supercritical Fluid Chromatography.

Further Reading

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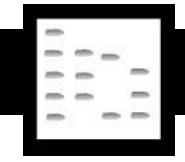
FOOD ADDITIVES



Liquid Chromatography

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Introduction

Food is a complex heterogeneous mixture of a wide range of chemical constituents such as moisture, carbohydrates, proteins, fibres, vitamins, etc. Besides these, processed foods contain a wide array of additives and contaminants. Analysis of product composition is a prerequisite for ascertaining product quality,

implementing regulatory enforcements, checking compliance with national and international food standards, contracting specifications and nutrient labelling requirements and providing quality assurance for use of the product for the supplementation of other foods.

Food preservatives form an important class of food additives. They are primarily used to prevent microbial growth, to improve or maintain the nutritional value of food, to maintain palatability and wholesomeness and to enchance flavour and colour. Other food additives used include colours, colour modifiers, flavours, flavour enhancers, humectants, non-nutritive sweeteners, pH control agents, thickeners, stabilizers and emulsifiers. Food additives are regulated and specified by law in most countries to ensure safety for the consumer and prevent deception and fraudulent practices. Labelling regulations require that information be provided on the kind of food, its processing and the additives contained in it.

The complex heterogeneous nature of foods demands effective separation techniques such as High Performance Liquid Chromatography (HPLC) with its wide array of column materials, and detectors. Food additives are usually present in small quantities in processed food items. Their separation from food constituents therefore requires a thorough understanding of the chemistry and physics of the food constituents and additives in order to select the best analytical procedures. Increased automation has gained universal acceptance for the effective separation and analysis of nearly all food components and food additives.

Typical HPLC Analytical Systems

In HPLC analysis of food additives, a single solvent (or solvent mixture) is often not sufficient to carry out the separation under isocratic conditions. Hence, solvent systems of varying proportions are generally used for gradient elution. Abundant literature is available on convenient, versatile and precise liquid chromatography (LC) separations of complex food constituents and additives. Problem areas such as band tailing, trace analysis, preparative separations and so on have received considerable attention and these particular problems can now be tackled in relatively systematic and simple ways.

LC is ideally suited for the separation of macromolecules and ionic species, heat-labile natural products and high molecular weight or less stable compounds such as proteins, nucleic acids, amino acids, dyes, synthetic polymers and food additives.

HPLC is well suited for the quantitative determination of food additives in one step and it has largely replaced other analytical methods.

On account of the wide range of different classes of chemical compounds and matrices encountered, it is not possible to give a universally applicable analytical scheme for food additives. **Figure 1** illustrates the various steps involved in the analysis of antioxidants in potato chips by HPLC as a typical example.

Preservatives

Preservatives are chemicals added to food products to prevent or inhibit the growth of microbes. Benzoic acid, sorbic acid, propionic acid and methyl-, ethyl- and propyl-esters of *p*-hydroxybenzoic acid (parabens) are the most commonly used preservatives.

Preliminary extraction from the food matrix before analysis is required. Steam distillation, solvent and solid phase extractions are the most commonly used methods. Hild and Gertz have reviewed the analytical methods available for the quantitative determination of preservatives in food.

For the HPLC determination of benzoates and sorbates, many methods have been reported using either isocratic or gradient techniques with RP-columns and UV detection at ambient temperatures. LC has been used to separate the homologous esters of *p*-hydroxybenzoic acid (parabens) including methyl- and propyl-hydroxybenzoates. This is of specific importance as the homologous esters of p-hydroxybenzoic acid (parabens) are a group of similar compounds having closely related properties. The method described can efficiently determine parabens along with BHA, TBHQ, PG, NDGA and Ionox-100 on reverse phase systems with electrochemical detection. The typical linear range extends from 10^{-11} to 10^{-6} mole of injected analyte. Recovery of parabens is about 80%. Detector potential for parabens is $+1.10$ V and the electrochemical detector provides low limits of detectability.

Normal as well as reversed phase methods have been used to determine the esters of p-hydroxybenzoic acid by extraction with acetonitrile. For normal phase HPLC, the use of LiChrosorb Si 60 columns with a mobile phase of iso-octane + diethyl ether + acetonitrile (500 + 35 + 0.3) and for RP HPLC, RP-18 columns with methanol–water (80 : 20) and UV detection have been suggested. Recoveries are 95–104% with 1–2% RSD. A method for the analysis of parabens in meat products has been developed in which the samples are extracted with acetonitrile, filtered and analysed on a C18 column. The peaks are detected and quantitated using UV detector at 254 nm. Average recoveries are 92% for methyl paraben and 94% for propyl paraben.

HPLC has been used to determine sorbic acid, benzoic acid and *p*-hydroxybenzoic acid (PHB) esters

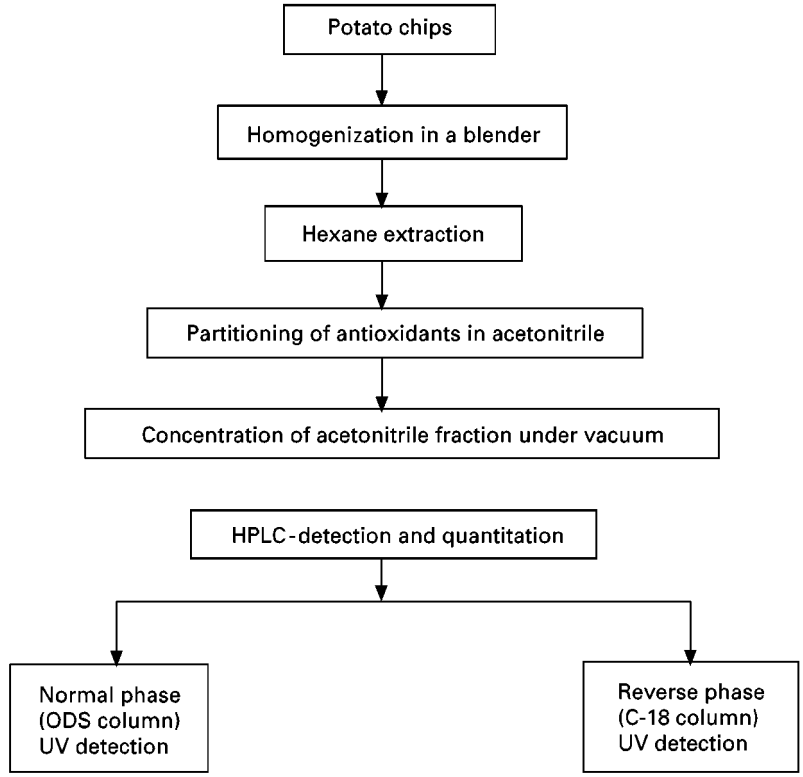
Potato chips
Homogenization in a blender
Hexane extraction
Partitioning of antioxidants in acetonitrile
Concentration of acetonitrile fraction under vacuum
HPLC-detection and quantitation
Normal phase (ODS column) UV detection
Reverse phase (C-18 column) UV detection

Figure 1 Stages of typical HPLC analysis of antioxidants in potato chips.

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in foods. A mixture of acetonitrile, 2-propanol, ethanol and oxalic acid was used for extraction. After refrigerating and separating the interfering materials by centrifugation, the extract was analysed without further cleanup, using Spherisorb ODS II (3 μm) and methanol–water–phosphoric acid–tetrahydrofuran as eluant and detection at 230 nm and 245 nm. The detection limits were 0.5 mg/kg, 2 mg/kg and 10 ng/kg for sorbic acid, benzoic acid and esters of p-hydroxybenzoic acid, respectively.

Using C-18 silica with methanol and phosphate buffer (1 : 9, v/v) a lower limit of detections ranging from 5 mg/kg to 1 mg/kg can be obtained for benzoic and sorbic esters of PHB.

The sorbate content of commercial yoghurt sample following ion-pair extraction of sorbic acid and benzoic acid in tri-n-octylamine has been reported. It uses a RP-18 column with methanol–phosphate buffer (40 : 60, pH 4.5, ionic strength 0.1). Mean recoveries are 70–88% with a precision of 1.1 to 3.3% RSD. Isocratic HPLC is suitable for the determination of the benzoic and sorbic acid in beer. Steam distillation and direct extraction as sample pretreatment methods for analysis of benzoic acid and sorbic acids in salad dressing mayonnaise have been compared. Benzoic acid in chilli sauce can be determined by using RP-HPLC with detection at 254 nm.

Gradient elution methods have been compared for the simultaneous determination of benzoic acid, sorbic acid and parabens in ground beef, non-meat products and pork sausages. These preservatives were extracted with 70% ethanol and analysed on a Novapak C-18 column with a linear gradient mobile phase consisting of 10–70% methanol in 1.5% aqueous ammonium acetate and 1.5% aqueous acetic acid over 10 minutes, with 10 minute hold. Recoveries of benzoic acid, sorbic acid, methyl-, ethyl-, and propyl parabens range from 99–103%. Seven preservatives have been simultaneously measured in 28 food samples. Better selectivity and sensitivity for HPLC compared to other procedures have been reported.

A method applicable to many liquid and solid foods has been described. A C-18 column is used with methanol–phosphate buffer (5 : 95, v/v) as a mobile phase. This work was carried out to check the specificity of the isocratic-LC method for common food additivies such as L-ascorbic acid, caffeine, artificial sweeteners, antioxidants and synthetic colours. The method is applicable for determining benzoic acid and sorbic acid in a wide variety of foods such as beverages, fruits, seafoods, vegetables, sauces, dairy products, bakery products and confectionery products. 4-Hydroxyacetanilide is used as internal standard and detection is at 227 nm. Mean recoveries of

90–105% with a precision of 1–6% and detection limit of 20 mg/kg have been achieved. RP-HPLC for quantitative and simultaneous determination of benzoic acid, sorbic acid, PHB, salicylic acid, 5-nitrofurylacrylic acid, *p*-chlorobenzoic acid and PHB esters in wines and beverages has also been reported.

Sulfites and sulfiting agents permitted in the food industry include sodium sulfite, sodium hydrogen sulfite, sodium metabisulfite, potassium metabisulfite, calcium sulfite and potassium hydrogen sulfite. In the 1980s, an ion-pair method was introduced for the determination of sulfites in fruit juices, dried bread, salad dressing, ground beef, liquid caramel, fruit cake, apple pie, raisin, apple juice, apple sauce, dried onions and white wine using high pressure ion-exchange (I-E) polystyrene with $-NH_3^+$ and $-NR_3^+$ groups as stationary phase and 0.0024 M Na_2CO_3 and 0.003 M Na_2HCO_3 as mobile phase with conductivity detector. It has a very good detection limit of 1 ppm and an analysis time of 25 minutes. The results were not affected by the presence of volatile acids or even organic sulfur compounds. Average recoveries of 98.3% with standard deviation of 1.88 were reported with a detection limit of 1 p.p.m.

Electrochemical detectors are now preferred for sulfite analysis due to their better sensitivity. Sulfites have been analysed in lemon juice, beer, mashed potato and white wines using anion exchange stationary phase and 6 mM H_2SO_4 as mobile phase with electrochemcial detectors. Average recoveries were 81–103% with standard deviation of 4.6%. Other food products analysed by different workers by this method include apple, avocado mix, broccoli, cabbage, ketchup, grape juice, mushrooms, onions and raisins.

Table 1 gives details of some methods for the analysis of preservatives in foods and food products.

Antioxidants

During storage, oils and fats undergo various reactions that reduce their nutritive value and also produce volatile compounds, to give unpleasant smells and tastes; the phenomenon is referred to as rancidity. In many cases the presence of antioxidants can inhibit the onset of rancidity.

Synthetic antioxidants permitted to be added to food are:

BHA – 1(or 3)-(t-butyl)-4-hydroxy anisole
PG – Propyl gallate
TBHQ – t-Butyl hydroquinone
NDGA – Nor dihydroguaiaretic acid
OG – Octyl gallate
DG – Dodecyl gallate
(BHT – Butylated hydroxytoluene is allowed in a few countries)

Table 1 Details of some methods for the analysis of preservatives in food and food products

Food Products	*Preservative analysed*	*Analytical details*		
		Stationary phase/ column	*Mobile phase*	*Detection system*
Meat samples	Parabens	μ-Bondapak C-18	45% acetonitrile in water	UV at 254 nm
Yoghurt	Sorbic acid, benzoic acid	RP-18	Methanol–phosphate buffer (pH 4.5, ionic strength 0.1) (40 : 60)	UV at 254 nm
Fruit ready to serve, beverages, jams, jellies, meat products	Benzoic acid, sorbic acid, parabens	Novapak C-18	Gradient elution of 10–79% methanol in 1.5% aqueous ammonium acetate and 1.5% aqueous acetic acid over 10 min	UV at 254 nm
Beverages, fruits, seafoods, vegetables, sauces, dairy, bakery and confectionery products	Benzoic acid, sorbic acid	C-18	Methanol : phosphate buffer (0.03 M, pH 6.5) (5 + 95)	UV at 227 nm
Salad dressing, caramel, fruit cake, apple pie, raisins, onions, sauce, white wine, fruit cocktail	Sulfites (free and total)	I-E polystyrene column	0.003 M $NaHO_3$, 0.024 M, Na_2CO_3, in pure water	Dionex conductivity detector

Satisfactory and complete extraction of antioxidants from a food matrix into various organic solvents is not always easy because of co-extraction of interfering substances. Antioxidants such as BHA, BHT, TBHQ and Ionox-100 are susceptible to losses due to evaporation and utmost care needs to be exercised during concentration under vacuum. NDGA, PG, OG and DG are relatively polar nonvolatile compounds and their recovery is usually satisfactory. HPLC produces good separation between chemically similar compounds in mixtures to be analysed and enables the determination of up to 15 different antioxidants in one single run.

The general analysis protocols for antioxidants in foods comprise extraction in solvents and determination by reversed phase HPLC. The best solvents for extracting antioxidants from fats are acetonitrile and water-alcohol mixtures. The fat is usually dissolved in hexane or petroleum ether and the antioxidant is then extracted into the polar solvent. The literature indicates the use of a variety of chromatographic procedures with UV detection at 280 nm as most commonly used. Mobile phases are acetonitrile, acetic acid, methanol and water.

A HPLC method for the simultaneous determination of phenolic antioxidants in vegetable oils, lard and shortening has been reported. It was concluded that nine antioxidants, viz, BHA, TBHQ, IONOX-100 and THBP, PG, OG, DH and NDGA in vegetable oils, lards and shortening could be separated by gradient elution with water–acetonitrile plus 5% acetic acid as mobile phase. The recoveries ranged from 96 to 103%. A rapid and specific HPLC method for analysis of TBHQ in vegetable oils is also documented. A HPLC method was investigated with amperometric detection to analyse BHA, BHT and TBHQ in edible oils. The antioxidants were well separated, identified and quantified with high sensitivity. Recoveries ranged from 98 to 101%.

The use of RP-HPLC to quantitatively determine five antioxidants – BHA, BHT, PG, OG and DG – in fats has been described. HPLC enables the determination of the full range of antioxidants from polar compounds to the non-polar substances in a single chromatogram using gradient elution. Sensitive detection wavelengths are at 280 nm for UV and at 315 nm for fluorescence emission measurements.

Amperometric detection, which is both sensitive and specific has been used. Determination of BHA and BHT in chewing gums after extraction in hexane and with a second extraction into dimethyl sulfoxide has been reported. The resulting extract was acidified with hydrochloric acid and separated on a μ-Bondapak C-18 column with a mobile phase of acetonitrile–water (55 : 45, v/v). Antioxidants and antimicrobials (Parabens) have been analysed in a variety of commercial products, such as cereals, snacks and shortenings using amperometric detection. The typical linear range is from 10^{-11} to 10^{-6} mole of injected analyte.

Seven antioxidants have been determined using a linear gradient from 30% solution B (acetonitrile–acetic acid 95 : 5, v/v) in solution A (water–acetic acid 95 : 5, v/v) to 100% solution B over 10 minutes with detection at 280 nm. Fifteen antioxidants have been measured in dried foods as well as fats and oils. The antioxidants were separated by isocratic elution with fluorescence and UV detection. Recoveries ranged from 80–106.7%.

The antioxidants diphenylamine and ethoxyquin were estimated using methanol–0.01 M ammonium acetate (60 : 30, v/v) with fluorescence and UV detection. This method has been used successfully for the separation of fungicide residues and antioxidants in fresh fruits. BHA, BHT, PG, OG, DG and TBHQ in corn oil, cottonseed oil and beef fat have been determined. A procedure for the determination of antioxidants in vegetable oils without prior extraction did not resolve BHT from neutral lipids and suffered from interference due to co-eluting materials. PG, trihydroxybutyrophenone, TBHQ, BHA, BHT, NDGA and 3,5-di-tert-butyl-4-hydroxy-methylphenol have been determined in fats, oils and dry foods. Antioxidants in dried foods such as potato flakes, dry coffee, whiteners and dessert topping mixes were isolated after rehydration and extraction in acetonitrile and subsequent separation on a C-18 column. The overall recoveries ranged from 64.3 to 103.6%. The method is highly accurate and hence was adopted as an official method (AOAC).

Tocopherols in vegetable oils have been separated by both reversed-phase and normal-phase LC. A method using a Radial PAK cartridge has been used for analysing individual tocopherols in eleven samples of lupine oil. The results showed the presence of γ-tocopherol (42–69 mg/100 g oil), and δ-tocopherol in traces (0.1–0.7 mg/100 g oil). This method is superior to GC in which up to 30% tocopherol losses occur during pretreatment of the sample. The simultaneous determination of α-tocopheryl acetate, tocopherols and tocotrienols in food involving extraction in hexane, separation on Lichrosorb Si-60 with hexane–di-isopropyl ether (93 : 3) as mobile phase and fluorescence detection at 290 nm, 330 nm, has been reported. Recoveries are 95–100% with a detection limit of ≤ 20 ng.

Most methods for the analysis of antioxidants use C_{18} columns with detection at 280 nm. However,

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electrochemical or fluorimetric detection or simultaneous detection by two or more techniques has also been used. Mobile phases are usually composed of aqueous acid (acetic/phosphoric acid), buffers or salts together with methanol or acetonitrile. In many cases results are improved by gradient elution.

A method using a C-18 column for α-tocopheryl acetate and tocopherols has been described which allows separation of nine synthetic phenolic antioxidants along with natural antioxidants. Gradient elution is with water-acetonitrile-methanol-isopropanol. This method not only allows simultaneous detection of antioxidants and triglycerides but is also useful in studying inhibition effects of antioxidants in oil.

BHA, BHT, TBHQ, NDGA and gallates have been resolved and quantitatively determined on a Lichrosorb RP-18 column with gradient elution using acetonitrile–water–phosphoric acid and detection at 280 nm. Fluorimetric detection can also be used. The analyisis of BHA, BHT, TBHQ and gallates in carrot juice, powdered milk, appetizers and cake using electrochemical detection has also been reported. It was suggested that as many as twelve antioxidants could be detected by a single isocratic HPLC analysis. The quantitation of BHA, BHT, TBHQ, NDGA, gallates and other antioxidants in foods using Supelcosil LC-18 column with acetic acid–water–acetonitrile as mobile phase and UV detector at 280 nm has also been documented.

Murakita (1992) and Klein & Leubolt (1993) have reviewed the analysis of various antioxidants by HPLC.

Table 2 shows details of major liquid chromatographic methods for the analysis of antioxidants in foods and food products.

Non-Nutritive Sweeteners

Saccharin, cyclamates, aspartame, acesulfame-K are some of the widely used non-nutritive sweeteners.

Soft drinks containing saccharin are readily analysed with minimal sample treatment. For juice, sweets, jams or desserts, an additional extraction step has to be performed. A method for the separation and detection of saccharin, sodium benzoate and caffeine has been reported involving the use of 5% acetic acid as mobile phase and UV detetion at 254 nm. The resolution factor was > 2.0 between saccharin and sodium benzoate and between benzoate and caffeine. The detection limits were 0.14, 0.05 and 0.024 μg for saccharin, benzoate and caffeine respectively. Analysis of non-artificially sweetened soft drinks gave no interfering peaks with these additives. This method has been adopted by the AOAC because of its accu-

Table 2 Liquid chromatographic methods for the analysis of antioxidants in foods and food products

Food products	*Antioxidant analysed*	*Analytical details*		
		Stationary phase/ column	*Mobile phase*	*Detection system*
Potato flakes	BHA, BHT	C-18	Reversed phase gradient elution by acetonitrile with 5% acetic acid and 5% acetic acid in water	UV, 280 nm
Coffee whiteners	TBHQ, BHA	C-18	Reversed phase gradient elution by acetonitrile with 5% acetic acid and 5% acetic acid in water	UV, 280 nm
Dessert topping PG, DG, OG mixes		C-18	Reversed phase gradient elution by acetonitrile with 5% accetic acid and 5% acetic acid in water	UV, 280 nm
Cheese, snacks, cake mix	BHA, BHT, TBHQ, PG, OD, DG	C-18	Reversed phase gradient elution by acetonitrile with 5% acetic acid and 5% acetic acid in water	UV, 280 nm
Oils, lards, shortenings	BHA, BHT, TBHQ, THBP, Ionox-100, NDGA	C-18	Reversed phase gradient elution. Water-acetonitrile with 5% acetic acid	UV, 280 nm
Instant cereals, snacks, gelatin desserts, hydrogenated fats	BHA, TBHQ, PG, NDGA and Parabens	μ-Bondapak C-18	Methanol–0.1 M ammonium acetate (or 0.01 M phosphate) buffer (1 : 1, v/v)	Amperometric detection

racy. An isocratic HPLC method using a cation exchange column, a 0.1 M ammonium dihydrogenphosphate mobile phase and UV detection at 214 nm has been reported for the detection of saccharin, aspartame, benzoic acid and caffeine in soft drinks. Base-line separations of these four additives were achieved. Changing the wavelength of detection from 254 nm to 214 nm led to an increase in the detection response of aspartame. At all levels of addition the recovery for aspartame was 100%. Analysis time could be reduced by increasing the flow rates without sacrificing resolution.

A gradient method for the separation of saccharin, aspartame, benzoic acid and some colours in soft drinks using a detection wavelength of 214 nm has been reported. The mobile phase was methanol (10% increasing to 60%) with 50 mM phosphate buffer at pH 3.6. For aspartame, either isocratic or gradient elution was used. A method for the determination of aspartame, cyclamate, dulcin and saccharin using an ion-pair separation with indirect photometric detection has also been reported. A method for determining acesulfame-K using UV detection at 237 nm and a mobile phase of water–methanol (9 : 1, v/v) containing 10 mM tetrabutylammonium hydrogensulfate has been reported. The absence of appreciable absorption above 200 nm by cyclamate has led to the advent of special methods for its detection. Post-column ion-pairing of cyclamate with either methyl violet or crystal violet renders it easily detectable. Pre-column derivatization agents used are sodium hypochlorite or *o*-phthalaldehyde. An ion pair HPLC method with indirect photometric detection of cyclamate has been used for thick yoghurt samples and solid foods such as biscuits.

A method has been described for the detection of acesulfame-K, saccharin, dulcin, benzoic acid, caffeine and vanillin in ready-to-serve beverages, dry beverage mix samples and other food products. The separation was carried out on a μ-Bondapak C-18 column using methanol–acetic acid–water (35 : 5 : 60, v/v/v) as mobile phase and with UV detection at 254 nm. This method is advantageous because all the additives can be detected in a single step, which renders it useful in routine food analysis. Biemer analysed acesulfame-K in candy gum using an anion exchange column, sodium carbonate (300 mg/L) as mobile phase and conductometric detection.

A method for the determination of aspartame, saccharin, benzoic acid, sorbic acid and caffeine in cola drinks, table-top sweeteners, soft drinks and complex foods on a LiChrosorb C-18, column using acetonitrile–0.1 M sodium dihydrogenphosphate (15 : 85, v/v) at pH 4.5 and UV detection at 215 nm has been reported. Analysis of acesulfame-K, alitame, aspartame, caffeine, sorbic acid, theobromine, theophylline and vanillin in table-top sweeteners, candy, liquid beverages and other foods using a μ-Bondapak C-18, column and a mobile phase of acetonitrile–0.0125 M potassium dihydrogen phosphate (10 : 90, v/v) at pH 3.5 and UV detection at 220 nm has been advocated. This method allows for the simultaneous determination of theobromine, theophylline, caffeine, vanillin, dulcin, sorbic acid, saccharin, alitame, aspartame and their degradation products in a single run of 60 min duration. Table-top sweetener, candy, soft drink, fruit juice, fruit nectar, yoghurt, cream, custard, chocolate and biscuits have been analysed by simple extraction or by just dilution using this method.

Some of the simpler LC methods for sweetener analysis are given in **Table 3**.

Food Colours

Colour is a prime sensory quality by which foods are judged and food quality and flavour are closely associated with colour. Consumers are conditioned to expect foods of certain colours and to reject any deviation from these expectations. Colourants also play a significant role in enhancing the aesthetic appeal of food. Colourants are very important ingredients in many convenience foods such as confectionery products, desserts, snacks and beverages.

The regulatory status of colourants used in different countries throughout the world is in a constant state of flux due to the toxicological considerations. An extensive review of genotoxicity of food, drug and cosmetic colours and other azo triphenylmethane and xanthan dyes has been published by Combes and Haveland-Smith (1982).

Synthetic colours can be classified by their chemical structure as azo (mono, di and tris), indol, triphenylmethane and methin dyes. They are mostly acidic or anionic and acidic groups like sulfuric acid, carboxylic acid or hydroxy groups form a negatively charged coloured ion. Basic or cationic dyes contain substituted amino groups.

The dyes have to be first extracted from the complex food matrix; adsorbents like wool fibres, powdered polyamide, cellulose ion exchange resins or RP cartridges (Sep-Pak C18) are frequently used. Ion-pair chromatography has been used for the quantitative analysis of twelve primary food colours in grape beverages with a mobile phase of 45 : 55 methanol–water, the useful detection wavelengths were 610 nm for blues and greens and 480 nm for reds, oranges

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Table 3 Simpler methods for the analysis of food products for non-nutritive sweeteners

Food products	*Sweetener analysed*	*Analytical details*		
		Stationary phase/ column	*Mobile phase*	*Detection system*
Coffee, carbonated cola, lemon beverages	Sucralose	RadialPak C-18	Water–methanol 70 : 30	RI
Fruit drinks, cherry nectar, mayonnaise, chocolate	Cyclamate	Nucleosil C-18	Methanol–water 80 : 20	UV, 313 nm
Fruit juice, yoghurt, Cola	Acesulfam-K	Lichrosorb RP-18	Methanol–water 9 : 1 with 1 mM tetra butyl ammonium hydrogen sulfate	UV, 237 nm
Cola, pudding, chocolate	Saccharin, cyclamate, Alitame	μ Bondapak C-18 or Supelcosil LC-18	Phosphate buffer 20 mM (pH 3.5) : acetonitrile 97 : 3	RI, UV 200 nm
Ready-to-drink and dry mixes of beverages, tomato sauce	Saccharin, Acesulfam-K	μ Bondapak C-18	Methanol–acetic acid–water, 35 : 5 : 60	UV, 254 nm
Candy, chewing gum	Saccharin, Acesulfam-K	AS4A, anion exchange resin	Sodium carbonate (140 mg/L)	Conductivity
Candy, beverages, pickles, soy sauce	Rebausides A and C, Stevioside	Lichrosorb NH_2	Acetonitrile–water	UV, 210 nm
Diet cola	Saccharin, Acesulfam-K	Hypersil ODS	Phosphate buffer (pH 3.5)–acetonitrile 85 : 15	UV, 216 nm
Soft drinks, candy, pickle	Rebausides A, Stevioside	Finepak SIL NH_2	Acetonitrile–water 80 : 18 with tetrabutyl-ammonium phosphate	UV, 210 nm
Fruit yoghurts	Aspartame	μ Bondapak C-18	Phosphate buffer 125 mM (pH 3.5)–acetonitrile 90 : 10	UV, array 400
Soft drinks, table-top sweeteners	Acesulfam-K, Sucralose, Saccharin, cyclamate	Supelcosil LC-18	Acetonitrile (20 mM) : phosphate buffer (pH 3.5)–gradient of 97 : 3 to 85 : 15	Vis 585 nM (after post column ion-pairing)
Candy, soft drinks, yoghurt, custard, fruit juice, nectar, biscuit, chocolate	Aspartame and its decomposition products, Saccharin, Alitame, Acesulfam-K	μ Bondapak C-18	Phosphate buffer 125 mM (pH 3.5)–acetonitrile 90 : 10, 85 : 15, 98 : 2	UV, 220 nm
Table-top sweeteners	Acesulfam-K	Lichrosorb RP-18	Potassium phosphate buffer	UV, 227 nm
Shrimp	Saccharin	IonPac AS-5	Sodium carbonate (7.7 mM) acetonitrile, sodium hydroxide (33 mM) Cyanophenol	Conductivity

and yellow. Ponceau, Fast red-E, Benzyl violet 4B, erythrosine and some non-permitted synthetic colours were separated. The procedure has been reported to be a viable and quicker alternative to TLC-spectrophotometric techniques. A method for the determination of L-orange, Sunset Yellow FCF and Ponceau 4R by means of ion-pair chromatography has also been described. It has been used for analysis of food dyes E 110, E 111 and E 12 in fish samples using Nucleosil, C-18 or Lichrosorb RP-8 columns and detection at 505 nm. The mobile phase consisted of water–acetone mixtures (80 : 20) with tetrabutylammonium chloride added as ion-pair agent (0.2 g/L).

Carotenoids in red bell peppers were separated without saponification using a C_{18} column and methanol–ethyl acetate as mobile phase with detection at 475 nm.

Table 4 Simpler methods for the analysis of colours in food and food products

Food products	*Colour analysed*	*Analytical details*		
		Stationary phase/ column	*Mobile phase*	*Detection system*
Fruit juices, grape beverages, confectionery	Ponceau, Fastred E, benzyl violet 4B, erythrosine	Ion-pair HPLC	Methanol–water	480 nm, 610 nm
Fish, bakery, meat products, miscellaneous products	E-110-orange II E-III-orange I and E-124 Ponceau 4R	Lichrosorb RP-8	Water – acetonitrile (80 : 20)	505 nm
Olive oil	Chlorophylls, carotenoids	C-18	Methanol – acetone	410 nm
Red bell pepper	Carotenoids	ODS	Methanol – ethylacetates (1 : 1)	410 nm
Strawberry	Anthocyanins	ODS	10–30% aq. acetonitrile with 0.5% TFA	Photodiode array detector

A rapid method has been reported for quantitation of chlorophylls and carotenoids in virgin olive oil, by solid phase extraction on a C-18 column. The fat free pigments were separated and concentrated. A total of 17 pigments were separated and quantitated with a C-18 column and gradient elution of water–ionic pairing reagent–methanol and methanol–acetone (1 : 1). Detection was at 410 nm and 430 nm. β-Carotene and other hydrocarbon carotenoids have been determined in red grape fruit cultivars with non-aqueous eluents using a C-18 column and isocratic mobile phase consisting of acetonitrile, methylene chloride and methanol (65 : 25 : 1, v/v/v).

Anthocyanins have been extracted from cultured cells of strawberry plants using a 35% solution of acetic acid–acetonitrile–water (20 : 25 : 55), containing 0.1% trifluoroacetic acid; using a C-18 column, and using 10 to 30% aqueous acetonitrile containing 0.5% of trifluoroacetic acid as eluent in 30 min at 40°C with photodiode array detection. The method yielded higher concentration of anthocyanin than other methods.

Table 4 gives details of methods for the analysis of colours in foods and food products.

Emulsifiers and Wetting Agents

Food emulsifiers assist the stabilization and formation of emulsions by reducing surface tension at the oil–water interface. Common food emulsifiers used are:

- lecithin and lecithin derivatives
- glycerol fatty acid esters
- hydroxycarboxylic acid and fatty acid esters
- lactylate fatty acid esters
- polyglycerol fatty acid esters
- ethylene or propylene glycol fatty acid esters
- ethoxylated derivatives of monoglycerides.

Quantitative analysis of emulsifiers is difficult as most of them are similar in structure, their commercial sources are quite heterogeneous and their extraction from starchy foods is very difficult. A key problem is the quantitative extraction of emulsifiers and the exclusion of interfering substances. This problem is further complicated by the presence of food ingredients such as proteins, and the innate heterogenicity of most of the emulsifiers as well as the wide variation in their composition. The schemes of analysis for lecithin, monoglycerides, TEMS, acetylated monoglycerides, partial polyglycerol esters, propylene glycol esters, polysorbates, lactic acid esters, ethoxylated monoglycerides and sugar esters have been discussed. Baur has recommended solvents for extraction of emulsifiers.

A method for the separation of monoglyceride (E 471), sodium stearoyllactylate (E 481), calcium stearoyllactilate (E 482), diacetyltartaric acid esters of mono- and diglycerides (E 472e) and mixed acetic- and tartaric acid esters of mono- and diglycerides (E 472f) on a semi-preparative column has been described. Various emulsifiers were identified by off-line high resolution mass spectrometer. Analysis of sodium or calcium stearoyllactrylate showed that the major components were 2-stearoyl and 2-palmitoyl lactic acid and their salts.

Sodium dioctylsulfosuccinate, a wetting agent, has been permitted in a variety of food products including dry beverage bases. A post-column ion-pair extraction method was employed using methylene blue as counterion. Then the compound was extracted into chloroform from the aqueous phase

DropBooks

For analysis, a CN column was used with acetone–0.01 M KH_2PO_4 (1 : 5, v/v) as a mobile phase.

See also: **II/Chromatography: Liquid:** Mechanisms: Reversed Phases. **III/Food Additives:** Thin-Layer (Planar) Chromatography.

Further Reading

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Thin-Layer (Planar) Chromatography

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Introduction

Thin-layer chromatography (TLC) is a relatively old technique among the other chromatographic separation methods. In food additive analysis, this simple technique is the tool of choice, mainly because the high throughput of samples that it can manage in parallel and the wide range of compounds that can be analysed simultaneously.

Food Additives

Anything added to food is not necessarily a food additive. Generally, a food additive is a substance or a mixture of substances different to the bulk of the food and present as a result of any aspect of production, processing or packing. This definition does not include hazardous contaminants.

The Codex Alimentarius Commission for Food Additives defines these as follows:

> **Food additive** means any substance not normally consumed as a food by itself and normally used as a typical ingredient of the food, whether or not it has nutritive value, the intentional addition of which to food for a technological (including organoleptic) purpose in the manufacture, processing, preparation, treatment, packing, packaging, transport or holding of such foods, results or may reasonably expect to result (directly or indirectly), in it or its by-products becoming a component of or otherwise affecting the characteristics of such foods. The term does not include 'contaminant' or substances added to the food for maintaining or improving nutritional qualities.
>
> Others definitions include:
>
> Substance with non-nutritive properties, known chemical composition, intentionally added to food; generally in small amounts, with the aim of improving presentation (appearance, flavour, texture) and conservation properties of foods.

In other countries, such as Spain, additives are all substances that can be added intentionally to food and drink, without the purpose of changing the nutritive value, to modify processing and conservation characteristics, as well as to improve their adaptation to the use for which it is produced.

Classification of Food Additives

Many methods have been used to classify food additives. The majority imply functional grouping.